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WHAT IS CLAIMED IS:

~~Patent Claims~~

1. A wave field microscope having an illumination or excitation system, which includes an illumination source, a first objective lens and a second objective lens, or a reflector, the first objective lens and the second objective lens, or reflector being so positioned with respect to one another that they are suited for generating a one-dimensional, standing wave field; having an object space, which includes holder and maneuvering device(s) for an object, and having a detection system, which includes an objective lens, an eyepiece, and a detector,

characterized in that

the illumination, i.e., excitation system includes, in two or all three spatial directions, at least one real or virtual illumination source for light beams, capable of coherence, and at least one reflector or beam splitter for decoupling beam components, or a further illumination source for light beams, capable of coherence, to each of which is assigned at least one objective lens, and which are each suited for generating light wave trains, the light wave trains of the one illumination source being aligned antiparallel or in variably adjustable angles to the light wave trains of the reflector, i.e., of the other illumination source, and in fact such that the light wave trains emitted by the one illumination source interfere with those of the reflector, i.e., of the other illumination source to form a standing wave field having plane wave fronts;

and that the detection system includes at least one detection objective lens, suited for epifluorescent detection, and/or at least one detection objective lens, which is suited for raster scanning point detection and

preferably has a high numerical aperture, and which is arranged with its optical axis normal to the wave fronts of one of the interfering wave fields, and which can be identical to one objective lens of the excitation system; with a flat (two-dimensional) detector, e.g., a camera, being arranged upstream from the detection objective lens suited for epifluorescent detection, and with the detection objective lens suited for raster scanning point detection having at least one stationary, confocal detection annular plate and/or aperture plate, and/or at least one stationary detection slit being arranged upstream from it, and with a point detector, in particular a photomultiplier, a photodiode, or a diode array being arranged downstream from it.

2. The wave field microscope as recited in Claim 1, characterized in that in at least one spatial direction, an objective lens of a low numerical aperture or a reflector is assigned to an objective lens of a high numerical aperture, and in one or both other spatial direction(s), either two objective lenses of a low numerical aperture or an objective lens of a low numerical aperture and a reflector are assigned to one another.

3. A wave field microscope having an illumination or excitation system, which includes an illumination source and an objective lens, which are so positioned with respect to one another that they are suited for generating a standing wave field, having an object space, which includes holder and maneuvering device(s) for an object, and having a detection system, which includes an objective lens, an eyepiece, and a detector,

characterized in that,

the illumination, i.e., excitation system includes, in at least one of the three spatial directions, at least one real or virtual illumination source for light beams, capable of coherence, and at least one beam splitter for decoupling at least one beam component, to which is assigned a common objective lens, into which the light beams, i.e., light wave trains of the illumination source(s) and of the beam splitter(s) can be launched in such a way that they produce on the rear focal plane (facing away from the object space) two spaced apart focal points, and that they run relatively to each other in a variably adjustable angle in the space between the two focal planes, and interfere to form a one-dimensional, standing wave field;

and that the detection system includes at least one detection objective lens, suited for epifluorescent detection, and/or at least one detection objective lens, which is suited for raster-scanning point detection and preferably having a high numerical aperture, which can also be identical to the objective lens of the excitation system, with a flat (two-dimensional) detector, e.g., a camera, being arranged upstream from the detection objective lens suited for epifluorescent detection, and with the detection objective lens suited for raster-scanning point detection having at least one stationary, confocal detection annular plate and/or aperture plate, and/or at least one stationary detection slit arranged upstream from it, and a point detector, in particular a photomultiplier, a photodiode, or a diode array arranged downstream from it.

4. The wave field microscope as recited in Claim 3,

characterized in that

the illumination, i.e., excitation system has in the same or in one of the two other spatial direction(s), in each case, at least one further real or virtual illumination source for light beams, capable of coherence, and/or at least one beam splitter for decoupling at least one beam component, to which is assigned in each case a further objective lens, through which the light beam(s) (light wave trains) are focused into the object space and are aligned in such away that they interfere with the light beams from the same or from the other or two other spatial direction(s), i.e., with the one or two-dimensional wave field formed by these, to form a two- or three-dimensional wave field.

5. The wave field microscope as recited in one of the Claims 1 through 4, characterized in that the object space includes an object mount fixture, in or on which the object is rotatably supported with the measuring structures, and/or, if indicated, with the calibration target(s), in the wave field, about one or two axes running orthogonally to one another, a rotational capability of about 360 degrees (2π) being preferred for at least one axis.

6. The wave field microscope as recited in one of the Claims 1 through 5, characterized in that the illumination source(s) producing the multi-dimensional wave field, and/or the reflector(s), and/or the beam splitter(s), and/or the objective lens(es) and, thus, the multi-dimensional wave field, are rotationally mounted about one or two axes running orthogonally with respect to one another.

7. The wave field microscope as recited in one of the Claims 1 through 6, characterized in that provision is made in the detection system for a scanner reflector, which is arranged so as to be suitable for forming an image of the lateral object regions with the desired, preferably maximal, fluorescence intensity.

8. The wave field microscope as recited in one of the Claims 1 through 7, characterized in that the illumination system includes in at least one of the three spatial directions, a real illumination source for the two- or multi-photon excitation, and in one or both other spatial direction(s), a real and/or virtual illumination source for the two- or multi-photon excitation, and that the standing wave fields ($WF_1, WF_2, \ldots, WF_i$) generated with it have wavelengths ($\lambda_1, \lambda_2 \ldots, \lambda_i$) ~~which~~ differ from one another, and have distances ($d_1, d_2, \ldots, d_i$) between their specific wave maxima or wave minima of $d_1 = \lambda_1/2n \cos\theta_1$ or $d_2=\lambda_2/2n \cos\theta_2$ or $d_i=\lambda_i/2n \cos\theta_i$ (where: n = the index of refraction in the object space, $\theta_1, \theta_2, \ldots\theta_i$ = the intersection angle of the light wave train of the wavelength $\lambda_1, \lambda_2 \ldots, \lambda_i$ with the optical axis), and with the wave fields WF_1, WF_2 … W_i being aligned in such away with respect to one another that at least a maximum of two or of all standing waves is situated at the same place (namely the location of a multi-photon excitation).

9. The wave field microscope as recited in one of the Claims 1 through 8, characterized in that an arrangement made up of an illumination source, objective lens, and an electrically conductive reflector, which is suited for generating a one-dimensional, electrical wave field, is provided relative to the object-carrier mount fixture,

and, in fact, so as to enable the measuring structures located in the object and/or calibration targets to be aligned through application of the electrical field - prior to or during the microscopic measuring operation.

10. A wave field microscopy method for DNA sequencing, with the use of a wave field microscope as recited in one of the Claims 1 through 9, characterized by the following method steps:

all complementary subsequences of the DNA sequence to be analyzed are produced in such a way that all subsequences begin at the same nucleotide of the sequence to be analyzed;

the fragments to be analyzed are all tagged at the 3' end with a reference fluorochrome label a and at the 5' end and/or at defined intermediate locations with a fluorochrome label a, g, c, or t – depending on whether the nucleotide base includes adenine (label a), guanine (label g), cytosine (label c) or thymine (label t) -, the fluorochrome labels a, g, c, t and a having different spectral signatures, and each containing one or a plurality of fluorochrome molecules;

the tagged DNA subsequences are fixed to a carrier in such a way they are present as a linear sequence, and are placed in a one- or multi-dimensional wave field microscope, with the linear DNA subsequences being so oriented with respect to the standing wave fronts, that a precise distance measurement (accuracy £1·10^{-10} m) can be implemented between a and a or g, c or t – once the intensity bary centers are defined and the imaging properties are calibrated -;

in that the signals of the fluorochrome labels are registered step-by-step, spectrally separated from one

another;

and from the distances of spacings between the fluorescent labels and their spectral signatures, the DNA base sequence of the DNA fragment to be analyzed is determined.

11. A calibration method for the multi-dimensional wave field microscopy as recited in one of the Claims 1 through 10, in which

before, during, or after preparing the object in question on or in an object holder, in particular a slide, object carrier fiber, object carrier capillary tube, or object carrier fluid, the object structures to be examined or to be localized - equivalent to the measuring structures - are labeled with fluorescent stains having different and/or the same spectral signatures, with such measuring structures to be localized, whose distance from one another is less than the width at half maximum intensity of the effective point spread function, being labeled with fluorescent stains having different spectral signatures;

with calibration targets of a defined size and spatial arrangement being labeled with the same fluorescent stains;

with the fluorescing calibration targets either being prepared together with the objects, i.e., measuring structures, or separately on or in the/ an object holder;

with the measuring structures and calibration targets being examined microscopically under identical conditions, simultaneously or sequentially;

and in the case of which, two defined calibration targets having different spectral signatures being measured at a time under consideration of the wavelength-

dependent imaging and localization properties of the particular optical system, with the measured values ascertained in the process - equivalent to the actual values - being compared to the previously known, actual distance values - equivalent to the reference values -, and from the difference between the actual values and reference values, a correction value - equivalent to the calibration value - being determined, which is used to correct the shift that is conditional upon the optical system, in the detection of various emission loci, in particular of the measuring structures,
characterized in that,

the biological object having the fluorochrome-labeled measuring structures, and/or the fluorochrome-labeled calibration target(s), is sequentially or simultaneously illuminated by individual (separate) standing wave fields, running orthogonally to one another in two or three spatial directions, and interfering with one another to form a two- or three-dimensional wave field, the fluorochromes being excited to emit fluorescence;

that to detect the fluorescence intensity, a camera and/or one or more two-dimensional arrangement(s) of individual detectors, each having a circular, annular, or slit-shaped plate, or an arrangement of a plurality of circular, annular, or slit-shaped plates is used;

that either the object having the measuring structures and/or the calibration target(s) or the one- or two-dimensional wave field, or both, is rotated during the measuring operation step-by-step, about one axis or about two axes running orthogonally to one another, the fluorochrome-labeled measuring structures and/or calibration targets being sequentially or simultaneously

illuminated by one or two individual standing wave fields disposed orthogonally to one another.